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CLAIMS

What is claimed is:

1. A method for identifying an agent that inhibits the catalytic activity of PTPbr7, comprising the steps of:

(a) combining, in a first cocktail, an amount of a PTPbr7, an amount of a substrate capable of being dephosphorylated by PTPbr7, and an amount of a test agent, in an assay buffer comprising a reducing agent;

(b) preparing a second cocktail comprising all of the ingredients of said first cocktail except for said test agent;

(c) incubating said first and second cocktails under conditions suitable to allow for a substantial amount of dephosphorylation of said substrate by said PTPbr7;

(d) terminating said incubation;

(e) quantitating the amount of dephosphorylation in each of said cocktails;

and

(f) comparing said amounts of dephosphorylation;

where a PTPbr7 inhibitor is a test agent whose presence results in less dephosphorylation than its absence.

2. The method as defined in claim 1 wherein said PTPbr7 is full length.

3. The method as defined in claim 2 wherein said PTPbr7 is recombinant rodent PTPbr7.

4. The method as defined in claim 3 wherein said rodent is a mouse or a rat.

5. The method as defined in claim 1 wherein said PTPbr7 is a fusion protein comprising the catalytic domain of PTPbr7 and a heterologous sequence.

6. The method as defined in claim 5 wherein said PTPbr7 is fused N-terminally to said heterologous sequence.

7. The method as defined in claim 5 wherein said PTPbr7 is fused C-terminally to said heterologous sequence.

8. The method as defined in claim 5 wherein said heterologous sequence is glutathione-S-transferase or a six-histidine residue tag.

9. The method as defined in claim 1 wherein said substrate is a small molecule mimetic.

10. The method as defined in claim 9 wherein said small molecule mimetic is para-nitrophenylphosphate.

11. The method as defined in claim 1 wherein said terminating is by adding a base to said assay buffer.

12. The method as defined in claim 11 wherein said base has a final concentration in said assay buffer of 0.95N NaOH.

13. The method as defined in claim 1 wherein said substrate is para-nitrophenylphosphate, a phosphoprotein, or a peptide having a phosphotyrosine residue.

14. The method as defined in claim 1 wherein said substrate is a phosphoprotein, or a peptide having a phosphotyrosine residue.

15. The method as defined in claim 14 wherein said peptide is [ENDpYINASL] or [DHTGFLTEpYVATRW].

16. The method as defined in claim 14 wherein said phosphoprotein is MAPK-P.

17. The method as defined in claim 1 wherein said suitable conditions for dephosphorylation are a temperature of from about room temperature to about 37°C, and an incubation time of from about 5 min to about 90 min.

18. The method as defined in claim 1 wherein said assay buffer comprises a buffer, a salt, and a detergent.

19. The method as defined in claim 18 wherein said buffer is Tris or Hepes, said salt is NaCl, and said detergent is Tween-20.

20. The method as defined in claim 19 wherein said buffer is 50 mM Tris or 50 mM Hepes, said salt is 150 mM NaCl, and said detergent is 0.05% Tween-20.

21. The method as defined in claim 1 wherein said reducing agent is DTT or BME.

22. The method as defined in claim 14 wherein said amount of said assay buffer is about 25  $\mu$ L, said assay buffer comprises about 50 mM Tris, about 0.15 M NaCl, about 5 mM DTT, and about 0.1% BSA, at about pH 7.4, said terminating is by adding a stop solution comprising malachite green dye, ammonium molybdate, and

- 5 Tween-20 to said cocktails, and waiting about 15 min, and said quantitating is by measuring the optical density at 620 nm of each of said stopped cocktails using a spectrophotometer, and comparing the optical density values with a set of standards comprising varied amounts of inorganic phosphate in said stop solution.

23. A pharmaceutical composition comprising an inhibitor of the catalytic activity of PTPbr7 as defined in claim 1 and a pharmaceutically acceptable carrier, vehicle, or diluent.

24. A method for treating a condition or disease in a mammal comprising administering to said mammal a pharmaceutical composition as defined in claim 23.

25. A method for identifying an agent that inhibits the catalytic activity of PTPbr7, comprising the steps of:

- 5 (a) combining, in a first cocktail, an amount of a PTPbr7, an amount of a substrate capable of being dephosphorylated by PTPbr7, and an amount of a test agent, in an assay buffer comprising a reducing agent;
- (b) preparing a second cocktail comprising all of the ingredients of said first cocktail except for said test agent;
- (c) incubating said first and second cocktails under conditions suitable to allow for a substantial amount of dephosphorylation of said substrate by said PTPbr7;
- 10 (d) quantitating the amount of dephosphorylation in each of said cocktails; and
- (e) comparing said amounts of dephosphorylation;

where a PTPbr7 inhibitor is a test agent whose presence results in less dephosphorylation than its absence.

26. The method as defined in claim 25 wherein said PTPbr7 is full length.

27. The method as defined in claim 25 wherein said PTPbr7 is recombinant rodent PTPbr7.

28. The method as defined in claim 27 wherein said rodent is a mouse or a rat.

29. The method as defined in claim 25 wherein said PTPbr7 is a fusion protein comprising the catalytic domain of PTPbr7 and a heterologous sequence.

30. The method as defined in claim 29 wherein said PTPbr7 is fused N-terminally to said heterologous sequence.

31. The method as defined in claim 29 wherein said PTPbr7 is fused C-terminally to said heterologous sequence.

32. The method as defined in claim 29 wherein said heterologous sequence is glutathione-S-transferase or a six-histidine residue tag.

33. The method as defined in claim 25 wherein said substrate is a small molecule mimetic.

34. The method as defined in claim 33 wherein said small molecule mimetic is O-methylfluorophosphate.

35. The method defined in claim 34 wherein said quantitating is by measuring the excitation at 485 nm and fluorescent emission at 535 nm of each of said cocktails using a fluorometer.

36. The method as defined in claim 25 wherein said suitable conditions for dephosphorylation are a temperature of from about room temperature to about 37°C, and an incubation time of from about 5 min to about 90 min.

37. A pharmaceutical composition comprising an inhibitor of the catalytic activity of PTPbr7 as defined in claim 25 and a pharmaceutically acceptable carrier, vehicle, or diluent.

38. A method for treating a condition or disease in a mammal comprising administering to said mammal a pharmaceutical composition as defined in claim 37.

39. A method of screening for an agent that inhibits the catalytic activity of PTPbr7, comprising the steps of: exposing cells or a cell line expressing PTPbr7 and capable of responding to NGF, to an amount of NGF, in the presence and absence of a test agent, under conditions suitable for a detectable NGF response to occur in the presence of a PTPbr7 inhibitor, detecting said response, and comparing said response, where a PTPbr7 inhibitor is a test agent whose presence results in more of an NGF response than its absence.

40. The method as defined in claim 39 wherein said cell line is a PC12 cell line, or a PC12 cell line stably transfected with a PTPbr7, and said cells are primary neurons, or primary neurons cotransfected with a cDNA clone of a PTPbr7 and lac Z.

41. The method as defined in claim 40 wherein said primary neurons are cholinergic neurons.

42. The method as defined in claim 41 wherein said cholinergic neurons are basal forebrain neurons.

43. The method as defined in claim 40 wherein said transfected PC12 cell line overexpresses said PTPbr7, and said primary neurons overexpress said PTPbr7.

44. The method as defined in claim 40 wherein said NGF response is neurite outgrowth.

45. The method as defined in claim 39 wherein said NGF response is survival.

46. The method as defined in claim 39 wherein said NGF response is the expression of any gene induced by NGF.

47. The method as defined in claim 39 wherein said NGF response is MAPK phosphorylation.

48. A pharmaceutical composition comprising an inhibitor of the catalytic activity of PTPbr7 as defined in claim 39 and a pharmaceutically acceptable carrier, vehicle, or diluent.

49. A method for treating a condition or disease in a mammal comprising administering to said mammal a pharmaceutical composition as defined in claim 48.

50. A method of screening for an agent that inhibits the catalytic activity of PTPbr7, comprising the steps of: exposing a cell line expressing PTPbr7 and capable of responding to NGF, to an amount of NGF, in the presence and absence of a test agent, under conditions suitable for detectable neurite growth to occur in the presence of a PTPbr7 inhibitor, detecting said neurite growth, and comparing said neurite growth, where a PTPbr7 inhibitor is a test agent whose presence results in more neurite growth than its absence.

51. The method as defined in claim 50 wherein said cell line is a PC12 cell line, or a PC12 cell line transfected with a PTPbr7.

52. The method as defined in claim 51 wherein said transfected PC12 cell line overexpresses said PTPbr7.